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RAPID COMMUNICATIONS

Plasma Levels of 8-Methoxypsoralen Determined by High-Pressure Liquid Chromatography in Psoriatic Patients Ingesting Drug from Two Manufacturers

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We have adapted a rapid and sensitive high-pressure liquid chromatographic technique (HPLC) to measure plasma levels of 8-methoxypsoralen (8-MOP) in 22 psoriatic patients receiving photochemotherapy with 8-MOP and long-wave ultraviolet light (PUVA). In this procedure, 1 ml plasma samples containing ammidin as an internal standard are extracted with benzene. After evaporation under nitrogen the residue is redissolved in methylenechloride:acetonitrile, 95:5, and chromatographed using a normal phase HPLC system with a 10 μ silica particle column and a UV detector at 254 nm. The sensitivity of the method is 10 ng/ml plasma. Plasma concentrations of 8-MOP were measured between 2 and 4 hr after ingestion of therapeutic doses of 8-MOP provided by 2 manufacturers. Mean 8-MOP plasma levels were 27±35 ng/ml plasma 2 hr after ingestion of the only drug presently available on the U.S. market, 8-MOP (Elder). These values were significantly below ($p < 0.001$) those obtained with 8-MOP (Roche) which were 104±79 ng/ml plasma. A number of patients on 8-MOP (Elder) did not have detectable levels of 8-MOP 2 hr after ingestion. The time course patterns also differed, possibly indicating a slower and less complete absorption for 8-MOP (Elder). Repeated time course studies in the same patient were reproducible although the absolute concentrations showed some variation. Preliminary evidence indicates that the plasma levels of 8-MOP have therapeutic relevance.

Photochemotherapy using 8-methoxypsoralen (8-MOP) combined with long-wave ultraviolet light (UVA) is called PUVA and has been used increasingly in recent years in the treatment of psoriasis. PUVA treatment of psoriasis is effective [1,2], but there is concern about long-term risks of its use. In order to avoid PUVA overdosage, and, perhaps equally important, underdosage, one must closely monitor UV doses and the amounts of available 8-MOP. The former is readily accomplished, but reliable and rapid routine methods for the determination of 8-MOP concentrations in body fluids have not been available. Previous workers have attempted to measure psoralen concentrations by various means including thin-layer chromatography [3,4], gas chromatography [5–7] and combinations [8,9]. High-pressure liquid chromatography (HPLC) offers advantages over other techniques. With HPLC a rapid, single step analysis can be performed measuring 8-MOP plasma levels with a few nanograms per ml. Normal [10] and reversed phase HPLC systems [11] have been used previously.

We have measured plasma levels of 8-MOP in psoriatic patients on PUVA therapy using HPLC with a microparticulate silica column. The pharmaceutical packaging properties of 8-MOP determine the resorbtion, the time course pattern and the peak blood levels of the drug. Quantitative blood levels with 8-MOP provided by different manufacturers might be expected to vary. One should not expect to be able to translate information obtained with one preparation directly to another. Furthermore, the interindividual variation in 8-MOP blood levels may be great [4,11,12] and comparisons between different preparations should be made in the same patient. Variation in 8-MOP levels on different occasions in the same patient has not been studied previously although this factor could also influence the therapeutic effectiveness.

MATERIALS AND METHODS

Patients

22 persons with severe psoriasis who were undergoing PUVA therapy were studied after informed consent had been obtained. There were 9 females and 13 males, ranging in age from 13–72 (mean age was 43 yr). Blood was drawn by venipuncture and collected in heparinized tubes. Plasma was separated and kept at −20°C until analyzed for 8-MOP. Blood samples were obtained 2 hr after ingestion of the 8-MOP drug (dose 0.5 −0.7 mg/kg body weight) at the time of the patient's UV treatment. In 11 patients blood was also collected at hourly intervals for 4 to 6 hr after administration of the drug. Time course studies were repeated from 2 to 4 times in 7 patients. Two preparations of 8-MOP were used: Oxsoralen provided by Paul B. Elder Co., Bryan, Ohio in 10 mg capsules; and Ro 21-4345 provided by Hoffmann-La Roche, Nutley, New Jersey in 10 mg and 40 mg capsules.

8-MOP Determinations

Psoralen standards: 8-MOP was obtained from Sigma Chemical Company, St. Louis, Missouri, and recrystallized twice from methanol. The purity was confirmed spectrophotometrically. Ammidin and 5-methoxypsoralen were gifts from AB Draco, Lund, Sweden, and 4,5′8 trimethylpsoralen was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. These agents were used without further purification. Structures of these psoralens are shown in Fig 1. All standard solutions were freshly prepared in a 95:5 mixture of methylenechloride: acetonitrile from 0.01% stock solutions of psoralens in methylenechlroide. All solvents used were analytical reagent grade.

Extraction: The extraction procedure was a modification of a technique described by Puglisi, de Silva, and Meyer [10]. To 1 ml samples of plasma were added: 200 ng of ammidin as internal standard; and 2.5 ml of a 1 M borate buffer (pH 9.0). Samples were extracted with 8 ml of benzene at room temperature on a reciprocating shaker for 20 min. After centrifugation for 15 min at 5°C the organic layer was transferred to another centrifuge tube and evaporated at 45°C under a stream of

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Abbreviations:
HPLC: high-pressure liquid chromatography
8-MOP: 8-methoxypsoralen, methoxsalen
UVA: long-wave ultraviolet light
PUVA: 8-MOP and UVA photochemotherapy

dry nitrogen. Immediately prior to the chromatographic analysis the dry residue was redissolved by agitating in 200 μl of the solvent phase (95:5 methylenechloride:acetonitrile).

All centrifuge tubes used for the extraction procedure were treated with a 2% solution of polyethylene glycol in chloroform for 5 min and heated in a drying oven at 150°C for 60 min.

Chromatographic procedure: A high-pressure liquid chromatograph (Varian 4100) equipped with a 254 nm UV-detector (LDC, Riviera Beach, Florida) was used for the analysis, which was adapted from Puglisi, de Silva, and Meyer [10]. The stationary phase was a microparticulate silica gel column (Partisil 10, Whatman Inc., Clifton, New Jersey) and the mobile phase was a 95:5 mixture of methylenechloride: acetonitrile. The system was equipped with a stop-flow injector, and the flow rate was 2.2 ml/min. A 20 μl aliquot of the redissolved sample was injected into the chromatograph.

Calculations: Determinations of the plasma levels were based on peak height measurements. A standard curve was prepared by adding increasing amounts of 8-MOP (10 to 400 ng/ml) to control plasma samples, each containing the same amount of the standard, ammidin (200 ng/ml), and plotting the 8-MOP/ammidin peak height ratio on a diagram (Fig 2). For statistical analysis Student's *t*-test was used throughout.

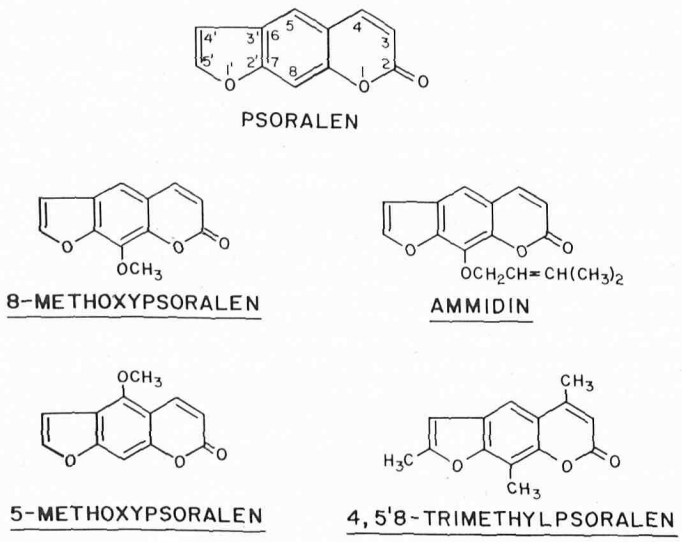

FIG 1. Chemical structures of psoralen, 8-methoxypsoralen, ammidin, 5-methoxypsoralen and 4,5'8-trimethylpsoralen.

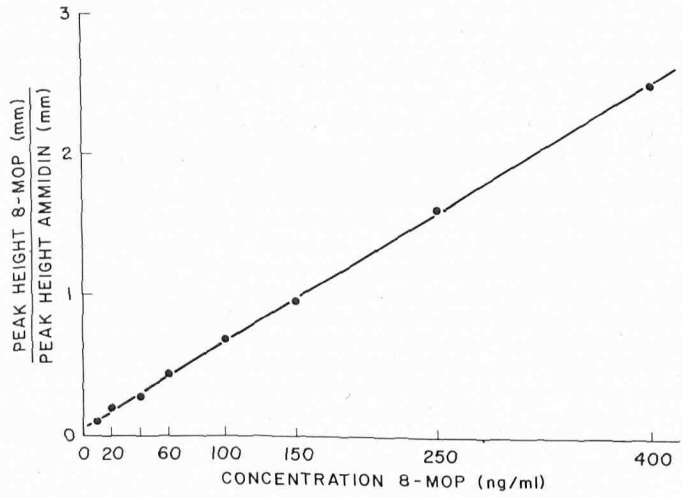

FIG 2. Standard curve for 8-methoxypsoralen using ammidin as internal standard. To 1 ml human plasma were added 200 mg ammidin and 10 to 400 mg 8-MOP and the psoralens were extracted with benzene and measured by HPLC as described in the methods.

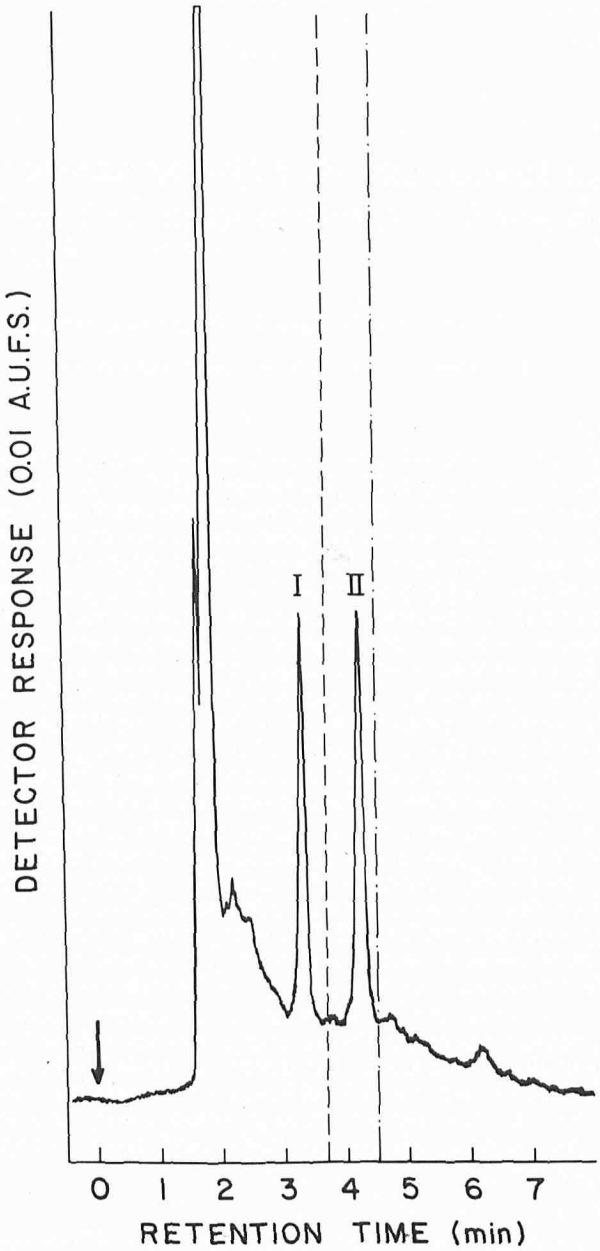

FIG 3. Chromatogram of extract from plasma collected 2 hr after the patient had ingested 50 mg 8-MOP. 200 ng of ammidin added to plasma sample. The point of injection is indicated by the *arrow*. *I*,Ammidin and *II*,8-MOP. The *dotted lines* show the retention times of 5-methoxypsoralen (---) and trimethylpsoralen (-·-·-) which were not present in the example shown in this figure.

RESULTS

With the present technique, plasma levels of 8-MOP of 10 ng/ml can be measured. The relationship between the peak heights of 8-MOP and ammidin was linear over a wide range of 8-MOP concentrations (Fig 2). A chromatogram of a sample of patient plasma with ammidin internal standard is shown in Fig 3. Ammidin is well separated from plasma impurities as well as from 8-MOP. In our system the retention times were: ammidin, 3.2 min; 5-methoxy-psoralen 3.7 min; 8-MOP 4.2 min; and trimethylpsoralen 4.5 min. Similar results to those with the Varian 4100 HPLC chromatograph have since been obtained by us with an Altex 330 system (Altex Scientific Inc., Berkeley, California).

The 2 hr plasma levels in different patients showed great variation, ranging from 0 to 268 ng/ml. The mean plasma level

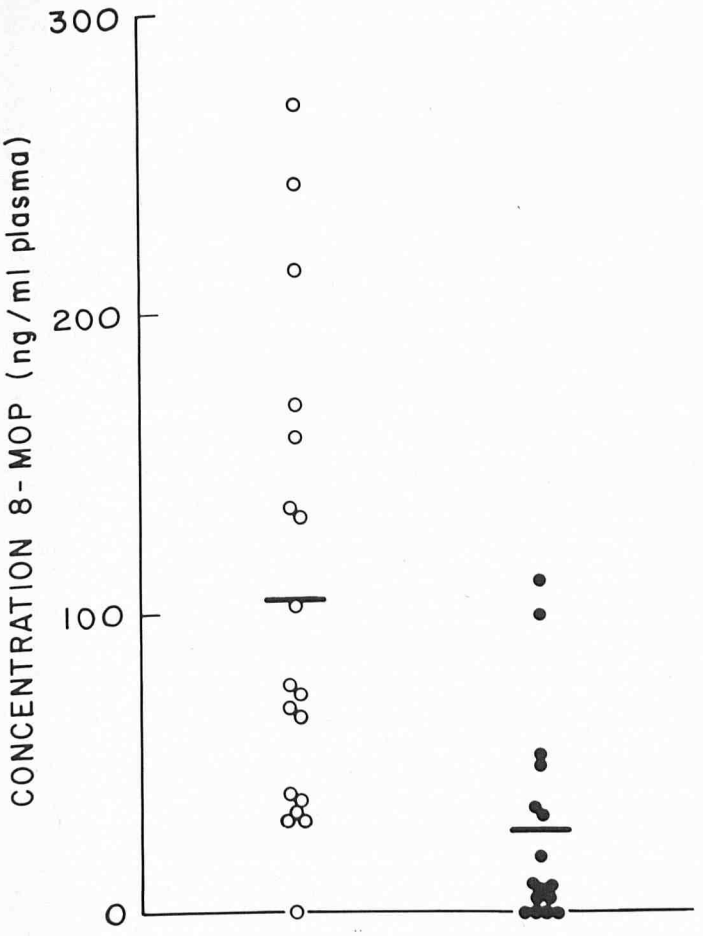

FIG 4. Plasma levels of 8-methoxypsoralen in PUVA patients 2 hr after drug ingestion. *Horizontal bars* indicate means ○,8-MOP (Roche); ●,8-MOP (Elder).

after 8-MOP (Roche) was 104 ng/ml (±79 ng/ml, n = 18) compared to 27 ng/ml (±35 ng/ml, n = 16) after 8-MOP (Elder) (Fig 4). This difference is statistically significant ($p < 0.001$).

Plasma levels as a function of time also revealed marked differences between the 2 drug brands. Patients receiving 8-MOP (Elder) usually did not reach the highest plasma level until 3 to 4 hr after drug ingestion. In contrast, patients on 8-MOP (Roche) showed a peak at 2 to 3 hr, and the maximal plasma levels were usually higher. Representative time course studies in 4 patients are shown in Fig 5. One patient, studied twice on each of the 2 drugs demonstrates this difference clearly (Fig 5*A*). When repeated in the same patient on different days, the time course was reproducible, although the absolute plasma concentrations varied somewhat (Fig 5*A* and *D*). The contents of several 10 mg capsules of 8-MOP (Elder) and 8-MOP (Roche) were each dissolved in 100 ml chloroform and filtered. Aliquots (10 μl) were diluted 1:100 in the solvent phase (95:5 methylenechloride:acetonitrile) after which 20 μl samples were subjected to HPLC as described. By this means we confirmed that the capsules of each manufacturer did contain 10 mg of 8-MOP.

DISCUSSION

The described HPLC analysis of 8-MOP after benzene extraction from plasma is a rapid and sensitive technique, and the detection limit of 8-MOP is comparable to other assays reported [5,9–11]. The use of HPLC eliminates the need for purification procedures, which may be necessary when gas chromatography is applied [5,9]. All glassware used for the extraction and evaporation procedures was treated with a 2% solution of polyethylene glycol which prevented uncontrolled losses such as psoralen binding to the glass. In our experience silanization of glassware as sometimes recommended [8] had an adverse effect.

The use of an internal standard added before the extraction of the plasma sample is of major importance in order to be able to account for variations in extraction efficiency between individual samples. As an internal standard we have chosen ammidin, another 8-substituted psoralen which has an HPLC retention time distinctly different from that for 8-MOP. Trimethylpsoralen and 5-methoxypsoralen, were also studied but were found to be less suitable as standard.

Extractions were initially performed from whole blood, serum and plasma. Chromatograms from serum or plasma were superior because they produced less chormatographic impurities, and 8-MOP concentrations in serum were about twice the concentrations of those in whole blood. Serum and plasma levels were almost identical (mean 95.3 ng/ml vs. 96.9 ng/ml, n = 16).

The wide range of 8-MOP concentrations found in our patients using the same drug (Fig 4) confirms other reports [4,11,12]. Many pharamceutical preparations of 8-MOP are in use in different countries and our results show that blood levels and time course relationships established for one 8-MOP drug brand may not necessarily hold true for another. Two hours after drug administration has been considered the optimal time for UV-A therapy because of maximal skin photoreactivity at this time [13,14]. Some workers, however, have reported maximal blood levels ½ to 1 hr after drug ingestion [12]. We measured plasma 8-MOP levels following ingestion of drug from 2 different manufacturers, and we found considerable differences between the 2 preparations in the blood levels at the time of UV-A treatment and in the time course relationships. Plasma concentrations 2 hr following 8-MOP (Elder) were significantly lower than those following 8-MOP (Roche). Some patients on 8-MOP (Elder) had no detectable plasma levels at the 2 hr point (Fig 4). This is partly due to the delayed time course seen with 8-MOP (Elder), many patients showing moderate and increasing plasma levels at 3 and 4 hr (Fig 5). The maximal levels attained during the 4-hr study were generally lower with 8-MOP (Elder), than with 8-MOP (Roche). Food intake has been shown to influence the plasma concentrations of 8-MOP

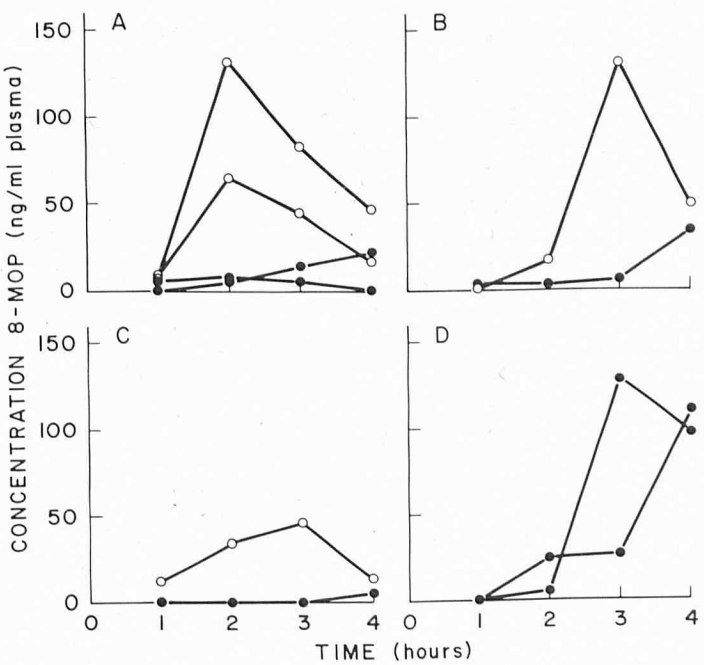

FIG 5. Repeated time course studies in 4 PUVA patients receiving either 8-MOP (Roche) (○) or 8-MOP (Elder) (●).

[8]. The patients we studied were accustomed to taking their drugs with a light meal to avoid nausea. We did not modify their food intake, preferring to keep this factor unchanged during our study.

Repeated time course studies in the same patient with the same drug showed a reproducible pattern over the 4-hr period of study although the absolute 8-MOP concentrations varied from one day to another. This has not been reported previously. In one patient who was studied twice on both drugs this consistency of time course patterns as well as the difference between the 2 drugs is clearly demonstrated (Fig 5*A*). Interdrug variations may be explained on the basis of differences in pharmaceutical formulation such as capsule and filler composition and perhaps size of 8-MOP crystals. Differences in actual 8-MOP content between 2 other proprietary drug preparations has also been reported [11]. Our preliminary data suggest that 8-MOP (Elder) and 8-MOP (Roche) do not differ in the amount of 8-MOP they contain.

In addition to differences between the drugs, interindividual variation also exists in patients receiving the same drug (Fig 4). This can be attributed to variations in rate and amount of absorption of 8-MOP from the gastrointestinal tract as well as to differences in the metabolic degradation of the drug.

A correlation between 8-MOP plasma levels and skin photosensitivity has not yet been demonstrated satisfactorily. Such a correlation cannot be studied on the basis of isolated 8-MOP blood levels at the time of phototesting [4], because PUVA therapy also depends upon inherent individual skin photosensitivity. The 8-MOP concentration and the skin sensitivity in combination should determine the amount of UVA energy required to evoke a phototoxic skin reaction in a given individual. This relationship should thus be studied by comparing simultaneous 8-MOP blood levels and skin phototests over a time period in the same patient. Such studies are now in progress in our department. It seems likely, however, that the bioactivity of 8-MOP correlates with blood levels. Several of our relatively unresponsive psoriatic patients promptly improved on PUVA therapy when irradiation time was changed from 2 to 3 hr after ingestion of drug in order to match the time of peak blood levels of 8-MOP (Elder).

8-MOP (Elder) is the only 8-MOP preparation commercially available in the U.S. today and these findings may thus have important consequences for the timing and dosage of PUVA therapy. Our results emphasize the importance of closely monitoring each of the components of PUVA therapy. Care should be taken to avoid undertreatment of patients receiving 8-MOP (Elder).

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